UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
NATIONAL VETERINARY SERVICES LABORATORIES
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SAM - 101

9 CFR 113.146	Rev. Sept. 1, 1981	Bovine Virus Diarrhea
Standard Requirement	Supersedes 2-01-79	Agent

SUPPLEMENTAL ASSAY METHOD

FOR

TITRATION OF BOVINE VIRUS DIARRHEA

VIRUS IN VACCINES

A. SUMMARY

This is an *in vitro* method which employs a cell culture system and an immunofluorescence procedure to determine the bovine virus diarrhea (BVD) virus content of vaccines.

B. MATERIALS

1. Cell Cultures. Tissue culture chamber/slides (8 chambers/slide) containing monolayers of bovine embryonic kidney (BEK) cells are used to titrate the BVD virus. Only cells found to be free of extraneous agents are used. (9 CFR 113.51 or 113.52)

2. Reference Virus. Veterinary Biologics' Reference BVD Virus is titrated as a control for the cell system.

3. Growth Medium & Diluent. Minimum essential medium (Appendix 1) is used for growth of cells and to make dilutions of the BVD reference and vaccine viruses.

4. Conjugate. Veterinary Biologics conjugated BVD specific-immune bovine serum is used to stain the cell monolayer.

5. Antisera. IBR and PI-3 specific-immune sera are used to neutralize these viruses.

C. METHOD

1. The vaccine to be assayed for BVD virus titer is rehydrated with the accompanying diluent.

2. In order to determine the BVD virus titer in combination vaccine products, it is necessary to neutralize the IBR and PI-3 viruses with specific-immune serum. This leaves the BVD virus fraction to be titrated.

a. Bovine Rhinotracheitis-Virus Diarrhea Vaccine, MLV, TCO, or CLO.

(1) The rehydrated vaccine is diluted 1:5 (1 + 4) with diluent.

(2) Equal volumes of the 1:5 dilution of vaccine and IBR specific-immune serum are mixed.

(3) The mixture is held at room temperature for 45 minutes to neutralize the IBR virus.

(4) This mixture constitutes a 10^{-1} dilution of the vaccine containing the BVD virus.

b. Bovine Rhinotracheitis-Virus Diarrhea-Parainfluenza-3 Vaccine, MLV, TCO, or CLO.

(1) The rehydrated vaccine is diluted 1:5 with diluent.

(2) One ml of the 1:5 dilution of vaccine is mixed with 0.5 ml of IBR specific-immune serum and 0.5 ml of PI-3 specific-immune serum.

(3) The mixture is held at room temperature for 45 minutes to neutralize the IBR and PI-3 viruses.

(4) This mixture constitutes a 10^{-1} dilution of the vaccine containing the BVD virus.

3. The cells to be used in this test are prepared as follows:

a. Cells are removed from the cell growth container (tissue culture roller flask or flat flask) by using a trypsin-versine solution (Appendix 2).

b. The cells are counted and diluted to 300,000 cells/ml with growth medium (Appendix 1) and are kept in suspension by using a magneti stirrer until ready for planting in the chamber/slides.

4. Serial 10-fold dilutions are made from the 1:10 dilution of the BVD vaccine virus and the reference virus with the diluent (growth medium), and each dilution is mixed by using a mechanical mixer.

5. The virus dilutions are used to inoculate the cell suspension by the "simultaneous inoculation" method as follows:

   a. Just before inoculation of cells with the virus dilutions, 0.3 ml of the cell suspension is measured into all chambers of each slide. Usually only enough slides are planted to accomodate 2 sets of virus dilutions at one time.

   b. One-tenth ml of each virus dilution is inoculated into each of 6 chambers containing the cell suspension.

   c. Non-infected cells serve as negative controls.

   d. The plastic lids are replaced and all of the chamber/slides can be gently agitated to mix and evenly disperse virus and cells.

6. The slides are incubated at 35 to 37 C in an atmosphere of 5% carbon dioxide and high humidity for 4 or 5 days.

7. The slides are removed from the incubator and can be examined for cytopathology, then processed for BVD fluorescence staining.

   a. The plastic lids and chambers are removed from the slides. The plastic chambers can easily be removed from the slide bonding material leaving the cell sheet on the glass surface.

   b. The slides are quickly rinsed in phosphate buffered saline (PBS) (Appendix 3), then in demineralized water and allowed to air dry.

c. These slides are fixed in acetone for 15 minutes, and again dried.

d. The cells are covered with conjugated BVD specific-immune serum and held in a high humidity 37 C incubator for 30 minutes.

e. Excess conjugate is washed from the slides in a gently circulating PBS bath for 10 minutes; then quickly rinsed in demineralized water and allowed to dry.

8. The cell monolayers are examined by fluorescent microscopy using a Ploem illuminator and blue-light (Xenon lamp).

a. The number of areas with fluorescing cells are recorded and the 50% endpoint calculated by the Reed-Muench or Spearman-Karber method.

b. The reference virus titer should be within 0.7 log of the established titer to be considered a valid test.

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Standard Requirement

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Bovine Virus Diarrhea
Agent

APPENDIX

1. Growth Medium

Minimum Essential Medium (MEM)

Edamin	0.5 %
MEM (Eagle) with Earles' salts* q.s. ad	100.0 %
L-Glutamine	1.0 %
Antibiotics - Gentamicin	50 mcg per ml
Amphotericin B	2.5 mcg per ml
Goat Serum, heat inactivated**	10.0 %

2. Trypsin-Versine Solution

NaCL	0.8 %
KCl	0.04 %
Na HCO_3	0.058%
Trypsin (Difco 1:250) (treated)	0.05 %
Versine (Disodium Salt)	0.02 %
Dextrose	0.1 %
Distilled H_2O q.s. ad	100.0 %

Sterile filtered and stored frozen

3. Phosphate Buffered Saline (PBS-Dulbecco)

NaCL	0.8 %
KCl	0.02 %
Na_2HPO_4	0.115%
KH_2PO_4	0.02 %

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* Available from Grand Island Biological Co., Inc. No endorsement expressed or implied.

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$CaCL_2$ (anhy.)		0.01 %
$MgCl_2$ $6H_2O$		0.01 %
Distilled H_2O q.s. ad		100.0 %